Renewable and Sustainable Energy Reviews 36 (2014) 256–269

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Environment-enhancing algal biofuel production using wastewaters

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ARTICLE INFO

Article history:
Received 4 January 2014
Received in revised form
14 April 2014
Accepted 27 April 2014
Available online 20 May 2014

Keywords:
Biofuel
Life cycle analysis
Microalgae
Sustainable development
Techno-economic assessment
Wastewater resources

ABSTRACT

The potential of algae-based biofuels to replace petroleum fuels and mitigate greenhouse gas production through microalgal photosynthesis has long been recognized. However, currently there are no commercial algae-to-fuels technologies that can overcome techno-economic barriers and address serious sustainability concerns. Coupling microalgae cultivation with wastewater treatment is considered as one of the most promising routes to produce bio-energy and bio-based byproducts in an economically viable and environmentally friendly way. This paper critically reviews the current status of this specific niche research area covering utilization of different types of wastewaters as media for algae cultivation, microalgae selection, bioreactor type, cultivation mode, environmental factors and operational parameters as well as harvesting techniques and production of a broad spectrum of biofuels and byproducts through various conversion pathways. Future development of practical solutions to key problems and integration of advanced algae cultivation and wastewater treatment, and system analysis approach to the evaluation of economic feasibility and sustainability of wastewater-based algal biofuel production are also discussed in depth.

Contents

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http://dx.doi.org/10.1016/j.rser.2014.04.073
1364-0321/

1. Introduction

Increasing greenhouse gas emissions, declining fossil resources, energy insecurity, and global warming issues are the major driving forces behind the search for alternative and sustainable renewable biofuels to meet the growing demand for transportation fuels [1–5]. Efforts to generate energy from biomass received considerable attentions during the 1970s in the United States because of the urgency to achieve energy self-sufficiency [6,7]. There has been renewed impetus in biomass energy since the mid-1990s due to the quest for ways to mitigate global climate change [8,9]. In recent years, U.S. Department of Energy (DOE) set an ambitious goal of replacing 30% of transportation fuel with biofuel and 25% of organic chemicals with renewable biochemicals by 2025 [10].

Biofuels derived from microalgae as viable third generation biofuels are promising alternatives due to unique characteristics inherent to algae such as fast proliferation, high oil accumulation, low water consumption rates, feasibility of growing on non-arable lands, tolerance to diverse environments, synergy with wastewater treatment, and capability of sequestering carbon dioxide (CO_2) through photosynthesis, etc. [11–18]. Moreover, it is believed that microalgae have the potential to generate an oil volume equivalent to over 17% of imports for the US transportation fuels and to meet the 2022 “advanced biofuels” production target set by Energy Independence and Security Act [19]. However, despite the fact that microalgae cells can be processed into a broad spectrum of advanced biofuels (Table 1) [2,5,20], many challenges have impeded the commercialization of algal biofuel technology. These challenges include the need for large amounts of freshwater, nutrients such as nitrogen (N), phosphorous (P), and trace elements in the current cultivation processes, lack of cost effective and energy efficient processes for algal biomass harvesting and oil extraction and conversion, lack of mature technologies for CO_2 mitigation via microalgae as well as system integration and evaluation, etc [5,15,17,21].

Coupling wastewater treatment with algae cultivation may offer an economically viable and environmentally friendly way for sustainable renewable algae-based biofuel and bio-based chemicals production since large quantities of freshwater and nutrients required for algae growth could be saved and the associated life cycle burdens could be reduced significantly [4,5,16,17,22,23]. For instance, algae can utilize nutrients such as N and P derived from a variety of wastewater sources (e.g., agricultural run-off, concentrated animal production operations, and industrial and municipal wastewaters), thus providing bioremediation while reducing treatment costs [4,5,16,24–27]. Moreover, they can also combine carbon-neutral fuel production with CO_2 sequestration from power plant or other emission sources, thereby providing an effective carbon capture and recycle opportunity, at the same time generating carbon credits [17,28]. Fig. 1 shows the simplified process diagram for advanced wastewater-based algae cultivation system with multiple benefits of water and nutrient recycling, biofuel and co-products production, wastewater remediation and reduced GHGs emission. Therefore, growing algae on waste streams offers many advantages over traditional algae farms.

Nevertheless, the challenges for wastewater-based algae cultivation system are also obvious, which include lack of understanding of

Table 1
Targeted bio-energy product associated with different conversion process using wastewater grown algal biomass as feedstock.

Final product	Conversion process	Reference
Biodiesel	Transesterification	[14]
Biohydrogen	Photosynthesis and/or fermentation	[128,129]
Green diesel, jet fuel and gasoline	Catalytic hydrothermal conversion	[2]
Acetone, butanol, bioethanol	Fermentation	[20]
Methane	Anaerobic digestion	[104]
Heat	Combustion	[130]
Crude oil and syngas	Thermochemical conversion	[95,96]

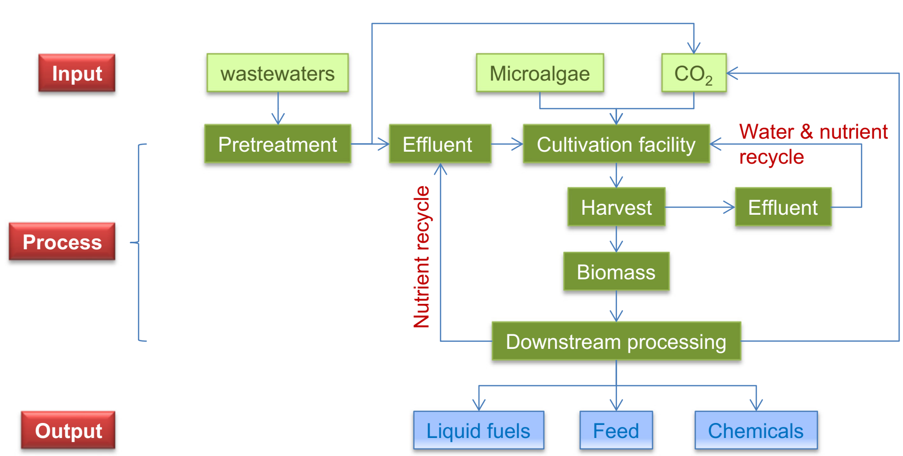

Fig. 1. Process diagram of cultivation of microalgae on swine manure wastewater.

the specific mechanisms of wastewater nutrients removal, lack of the robust microalgae strains that could tolerate different types of wastewaters and environmental stresses, culture collapse risks due to contamination of microorganisms such as bacteria, virus, fungi, or grazing and invading microalgae species, low algal biomass productivity and lipid content, lack of high efficiency and low-cost downstream processing, and lack of systematic analysis of economic feasibility for such cultivation system, etc. All these must be overcome through scientific breakthroughs and innovation.

The advantages and challenges of algae-based biofuel production were discussed in a number of papers, few of which dealt with wastewater-based algae cultivation systems especially for biofuel and bio-based chemicals applications. This review article is primarily focused on wastewater-based algal biofuel production and provides comprehensive information on past algal research, recent advancements, and technical challenges critical to algal production and economic and environmental impacts. Finally, further research and development is proposed.

2. History of wastewater-based algal biofuel research

Cultivation of algae on wastewaters evolved from the use of algae in wastewater treatment. The first research on using microalgae for wastewater treatment started as early as the 1950s in California, USA [29]. In that study, the symbiotic algal–bacterial relationship in waste stabilization pond was proposed in which algae were used as tiny aeration devices to provide large amount of oxygen (O_2) through photosynthesis for aerobic bacteria to oxidize and degrade the organic compounds in wastewaters while heterotrophic bacteria concomitantly release CO_2 and the nutrients needed by microalgae during photosynthesis [29,30]. The nutrients were removed efficiently in such a symbiotic system. While the initial purpose of the algae-based pond process was to further treat the secondary effluent before discharge to water bodies in order to avoid eutrophication [31-34], it was demonstrated that algae-based wastewater treatment could remove the nutrients (e.g., N and P) from settled domestic sewage more efficiently than traditional activated sewage process [31,35], indicating a great potential of algae-based wastewater treatment system.

The concept of growing algae on wastewaters for production of biodiesel at cost competitive to petroleum-based diesel was first proposed and discussed in the close-out report for the Aquatic Species Programs (ASP) supported by the U.S. DOE [11], which clearly suggested that the key point for the economic feasibility of algal biofuel production is combining algal cultivation with wastewater treatment. It is worth noting that, apart from obligate photoautotrophic growth, numerous studies have shown that some facultative heterotrophic microalgae species could utilize organic-rich wastewaters to stimulate fast growth as well as obtain high algal biomass productivity and lipid productivity [4,5,15–17,22,26,36]. The presence of both organic and inorganic carbon sources in wastewaters supports trophic conversion of some facultative heterotrophic microalgae species through mixotrophic/heterotrophic growth mode. Above growth mode may have many advantages over traditional obligate photoautotrophic growth mode, including: (1) faster growth and higher productivity [26,36]; (2) lower light requirement [37]; (3) efficient contaminant removal rate [17,26]. Mixotrophic growth conditions are believed to increase uptake rates of ammonium and the expression of nitrogen assimilation enzymes significantly; for example, when acetate was added to autotrophic *Scenedesmus obliquus* medium and cultivated under mixotrophic condition, the rate of ammonium uptake is four times higher than that occurring in autotrophic condition (17.8 mmol cell^{-1} min^{-1}) [38]; and (4) lower harvest and downstream processing costs due to higher biomass density in the culture broth especially for organic carbon-rich wastewaters [16,17]. The algal biomass productivities of over 30 g/m^2/d grown on organic-rich municipal and animal wastewater have been reported in literature [26,37,39]. In addition, the strategies to combine the advantages of different metabolic modes have been developed to regulate the algal biomass composition for different purposes or target products. For example, Oyler [40] developed a process of sequential photoautotrophic and heterotrophic growth (PHM) for algal biofuel production. A photoautotrophic–mixotrophic two-phase culture model (PMM) for algae-based biodiesel production using glycerol, glucose and sucrose as organic carbon was studied and discussed by Das et al. [41]. A similar photoautotrophic–heterotrophic culture mode (PHM) was also developed mainly focused on high algal cell density production [42]. Recently, Zhou et al. [17] developed a hetero-photoautotrophic culture mode (HPM) to effectively couple treatment of organic-rich wastewater such as concentrated municipal wastewater (CMW) with enhanced nutrient removal and low-cost biofuel production, which may be the future direction of wastewater-based algae cultivation system [36,43,44].

3. Wastewater resources for algal biofuel production

Algae can grow in various aquatic environments, such as fresh and marine water [45], municipal wastewaters [4,16,32,46], industrial wastewaters [25] and animal wastewaters [5,26,27,43,44] as long as there are adequate amounts of carbon (organic or inorganic), N (urea, ammonium or nitrate), and P as well as other trace elements present. Wastewaters are unique in their chemical profile and physical properties as compared with fresh and marine waters. Recent researches indicated the great potential of mass production of algal biomass for biofuel and other applications using wastewaters [4,16,17,25,26,46,47]. However, wastewater-based algae cultivation still faced with many uncertainties and challenges including variation of wastewater composition due to source, infrastructure, weather conditions, and pretreatment methods, improper nutrient ratios (e.g., C/N and N/P), high turbidity due to the presence of pigments and suspended solid particles which affects light transmission, and the presence of competing microflora and toxic compounds, and accumulation of growth inhibiting compounds which is worsened if water is recycled and reused. Growing algae in municipal and agricultural wastewaters have been extensively studied probably because the municipal and agricultural wastewaters are widely available and are less variable than other types of wastewaters (e.g., industrial wastewaters) [4,16,17,22]. Therefore, the following sections will be focused on algae growth in and nutrient removal from municipal and agricultural wastewaters.

3.1. Municipal wastewater

The typical process flow in municipal wastewater treatment plant is shown in Fig. S1. In general, four different types of wastewater streams are generated in different stages, including wastewater before primary settling, wastewater after primary settling, wastewater after activated sludge tank and concentrated municipal wastewater generated during sludge centrifuge, also called “centrate”. Wang et al. [46] investigated the growth of *Chlorella* sp. on above four different types of wastewater for their abilities to utilize and remove N, P, COD, and other trace elements and concluded that algae growth profile and nutrient removal efficiencies were proportional to the nutrient concentration of municipal wastewaters derived from different process stages of municipal wastewater treatment plant. It was found that the algal growth was significantly enhanced (more than 10 times higher) in

the centrate wastewater probably due to its much higher levels of COD, N, and P compared with other wastewater streams [46]. Similar research was conducted by Li et al. [15] to evaluate the feasibility of growing *Chlorella* sp. on centrate wastewater and the results showed that the algae removed ammonia, total N, total P, and COD as high as 93.9%, 89.1%, 80.9%, and 90.8%, respectively. Further scale-up experiments in semi-continuous operation mode showed that the daily biomass productivity reached 0.92 g L^{-1} d^{-1} probably also attributed to the high level of nutrients in this type of wastewater. Zhou et al. [16] further demonstrated that *Auxenochlorella Protothecoides* UMN280, isolated from local municipal wastewater treatment plant, could reach a net biomass productivity of 1.51 g L^{-1} d^{-1} when cultivated in a 25 L coil bioreactor with semi-continuous operation at optimal hydraulic retention time of 3 days, which is much higher than that grown in municipal wastewater with low nutrient levels (9.2 mg L^{-1} d^{-1}) [48]. All above studies suggested that growing algae in nutrient-rich municipal wastewater was a new option to enhance algal biomass productivity and serve the dual roles of nutrient reduction and cost-effective biofuel feedstock production.

3.2. Agricultural wastewater

US agricultural activities generate thousands of millions tons of wastewaters each year, especially in the animal production sector. In the US, already in 1997, 60% of the total recoverable nitrogen and 70% of total recoverable phosphorus produced as manure exceeded the capacity of the producing farm to directly use manure as fertilizer for crops and pasturelands. A comparison of the mineral composition of several classic mass culture media and animal manure wastewaters shows that animal manure wastewater appears to be a suitable medium for the growth of microalgae [26,27,49,50].

Numerous researches reported that microalgae are efficient tiny cell factory for removing N and P from manure-based wastewater [5,26,27,43,44,51–53]. For example, the green alga *Botryococcus braunii* grew well in swine manure wastewater containing 788 mg L^{-1} NO_3 and removed 80% of the initial NO_3 content [51]. Studies of nutrient recovery from dairy manure using benthic freshwater algae have been considered to be very effectively due to the significantly higher nutrient uptake rates in some species of benthic algae than those in planktonic suspended algae [52,54,55]. These species include *Microspora willeana*, *Ulothrix* sp. and *Rhizoclonium hierglyphicum*. Singh et al. [56] evaluated the potential of growing mixotrophic microalgae on digested poultry litter effluent and found the maximum algal biomass productivity of 76 mg L^{-1} d^{-1} with the relatively low lipid content (less than 10%) and high protein (39%) and carbohydrate (22%) content could be achieved, suggesting that the harvested algal biomass from poultry litter effluent could be ideal feedstock as animal feed supplement. Kim et al. [57] developed a low-cost media in which deep seawater was mixed with fermented swine urine and cow compost water for enhanced algal biomass production, suggesting the great potential of the nutrient-rich animal manure wastewater to stimulate fast algae growth through nutrients supplement.

However, there are some major issues when animal manure wastewater was used for algae cultivation, which include: (1) high turbidity due to presence of solid particles, which would affect light penetration significantly; (2) high nutrient concentration especially high ammonia concentration (Table S2), which could inhibit algae growth considerably; (3) a large portion of the carbon sources is locked in the large insoluble organic compounds and unavailable for algae to assimilate; (4) a large quantity of freshwater is necessary to dilute the concentrated animal wastewater unless water recycling and reuse is enabled; and (5) high performance algae strains adapted to the adverse environment in animal wastewaters have not yet been developed.

In order to address above issues, numerous methods and strategies were developed and adopted. For example, a study by Mairtin et al. [58] suggested that the use of hyperconcentrated algal biomass at initial inoculums grown on diluted concentrated pig manure (20–100 fold dilution) is a promising way to obtain dual purpose of simultaneous swine manure treatment and protein-rich algal biomass production. Moreover, after proper dilution, the initial ammonia concentration of animal manure wastewater was in optimal range and did not inhibit algae growth any more during cultivation. However, after high dilution, the soluble organic and inorganic carbon was insufficient to support continuous algae growth [26,27]. Wang et al. [26] demonstrated that 20-fold dilution was the best for fast algae growth with maximal algal biomass productivity using dairy wastewater. Woertz et al. [59] reported that the volumetric productivity of 17 mg/day/L of algal biomass concentration and lipid content ranged from 14 to 29% were achieved when growing green algae on dairy wastewater supplemented with CO_2. Supply of exogenous CO_2 as inorganic carbon may be beneficial for enhanced algae biomass productivity and improved nutrient removal [17,60,61]. The algae growth mode (autotrophic, mixotrophic or heterotrophic) is another key factor affecting algae biomass yield and nutrient removal when grown on high organic strength wastewater like diary and swine manure wastewater. Recently, Zhou et al. [17] and Hu et al. [44] analyzed the organic profile of both raw and digested swine manure and found that the major substances in the swine wastewater were sugar, acetic acid, propionic acid, and butyric acid, which are ideal organic carbon sources for some facultative heterotrophic strains to be utilized for fast growth [4,17,43,44]. Moreover, an acidogenic digestion of swine manure methods was developed to improve volatile fatty aicds (VFAs) significantly (mainly acetic, propionic and butyric acid) [43,44], which provided a new option for enhanced algal biomass productivity and improved nutrient removal for animal manure.

Overall, screening facultative heterotrophic microalgae strains which could adapt well in various wastewater environments, developing effective pretreatment for enhanced VFA profile in wastewaters and efficient cultivation system seems to be the most promising ways for animal wastewater remediation and maximal algal biomass production as biofuel feedstock.

3.3. Industrial wastewater

Due to the variable constituents of wastewaters from different industries, cultivation of microalgae on this type of wastewater may face many additional challenges. Among them, the potential impact of toxic compounds (e.g., some heavy metals) present in many industry wastewaters on microalgae growth is significant. Currently, research on algae-based treatment is focused mainly on the remediation and removal of heavy metal pollutants (cadmium, chromium, zinc, etc.) and organic chemical toxins (hydrocarbons, biocides, and surfactants) rather than algal biomass accumulation for biofuel purpose [62–64]. Although industrial wastewaters are commonly considered unsuitable for algae cultivation due to their intrinsic properties of relatively unbalanced nutrient profile and high toxic compounds, a few studies demonstrated the potential of microalgae grown on different industrial wastewaters for algal biomass production. For example, wastewater from carpet mill effluent contained process chemicals and pigments used in the mills, plus a range of inorganic elements including low concentrations of metals, and relatively low concentrations of total P and N. This type of wastewater was shown to be low enough in toxins and high enough in P and N to support the growth of two freshwater microalgae *B. braunii* and *Chlorella saccharophila*, and a marine alga *Pleurochrysis carterae* [25]. With the very large amount of wastewater available from this industry, a significant

amount of biomass and potentially biodiesel could be generated from this resource. Recently, OriginOil Inc. [65] claimed that a breakthrough algae-based technology that could help clean up wastewater generated in oil well water flooding and hydraulic fracturing was successfully developed. According to the U.S. Department of Energy, a significant amount of water is produced daily as a waste stream from onshore drilling of oil and gas. For every barrel of oil produced globally, an average of three barrels of contaminated water is produced [66]. An estimated 56 million barrels of water are generated every day as a waste during the onshore oil and gas production in the United States [67]. If all these wastewaters could be used for algae cultivation, approximate 0.7 million gallons of microalgal oil is expected to be produced every day based on 1 g/L microalgae concentration and 30% lipid content, which may contribute a small fraction of existing demand for transport fuels in US. It should be noted here that there is another type of industrial wastewater, which named food industrial wastewater (e.g., wastewater derived from olive-oil pretreatment, molasses wastewater, etc.), which is not in the same categories of those mentioned industrial wastewaters above [47,68]. For example, the highest growth rate of $0.044\ h^{-1}$ could be achieved in the culture of 5% food industrial wastewater from olive-oil extraction and biomass productivity could be improved significantly in the culture with 100% above industrial wastewater [68].

In summary, the “best wastewater”, which is able to provide optimal nutrient profiles similar to those of commercial artificial media for the selected robust microalgae species to achieve maximal algal biomass productivity and efficient nutrient removal, does not exist. Low nutrient concentration for the targeted wastewater is one of the key factors influencing the final algal biomass and lipid productivity. Therefore, the chemical characteristics of wastewaters should be analyzed and evaluated in more details prior to use as media for microalgae cultivation. A detailed comparison of the nutrients profiles among different types of wastewaters is shown in Table S2. It is apparent that wastewaters derived from animal manure and wastewaters generated from the activated sludge thickening process have the characteristics of rich nutrients including high concentration of phosphorus, ammonium, nitrogen and COD. While from the point of view of ratio of C/N and N/P for these wastewaters, the centrate municipal wastewater was considered the best media to support the fast algae growth [4,15,16,36,46]. One option to address this issue is to mix two and/or more types of wastewaters with proper ratio in order to obtain the optimal nutrients profiles so as to balance N/P and C/N ratios for fast algae growth and at the same time for efficient treatment of wastewater.

4. Wastewater-based algal production technologies

Growing microalgae on different types of wastewaters (e.g., agricultural run-off, concentrated animal feed operations, and industrial and municipal waste streams) has been studied over

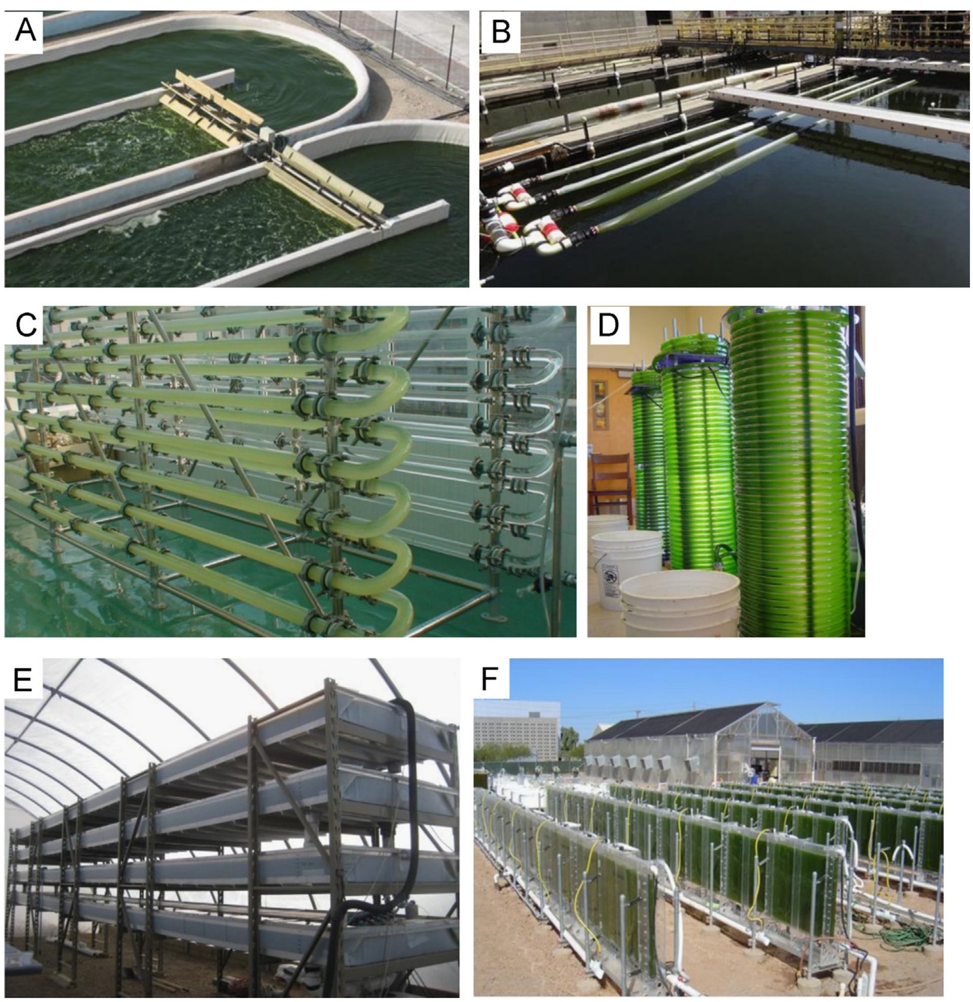

Fig. 2. Reactor configuration for microalgal cultivation: (A) raceway pond (from Sapphire Inc, America); (B) floating photobioreactor (from OMEGA system-NASA; (C) tubular bioreactor (from center for biorefining, University of Minnesota); (D) coil bioreactor (from center for biorefining, University of Minnesota); (E) multi-layer bioreactor (from center for biorefining, University of Minnesota; (F) flat-panel Bioreactor (from Nanovoltaics technologies, America).

the past decades. The success of such studies heavily depends on the performance of the selected microalgae strains. Many microalgae species such as *Chlorella* sp., *Scenedesmus* sp., *Micractinium* sp., *Actinastrum* sp., *Heynigia* sp., *Hindakia* sp., *Pediastrum* sp., *Chlamydomonas* sp., *Dictyosphaerium* sp., *Botryococcus* sp. and *Coelastrum* sp. have been tested and were proved to be able to utilize and remove N and P as well as other trace elements in the wastewaters [4,16,26,29,31,32,51,59,69–71]. Moreover, the harvested low-cost algal biomass could be used as an ideal feedstock for production of biofuels (Table 1) and other value added byproducts such as drugs, foods, fertilizers, and animal/fish feed supplements [27,72,73]. All these studies will be of great importance to the development of wastewater based microalgae cultivation system for above applications.

4.1. Strains

Microalgal strains are generally sensitive to different types of wastewaters due to the imbalance in nutrient profile, deficiency of some important trace elements, and presence of inhibiting/toxic compounds in wastewater streams, and only limited number of strains within a few species (e.g., *Chlorella* sp. and *Scenedesmus* sp.) could adapt well in different wastewater environments [4, 15–17,25,31,32,35,36]. There is a great need to select more robust microalgal strains that are tolerant to specific type of wastewater of interest. Numerous researches demonstrated that microalgae adapted to culture conditions similar to where they were found and generally grew better than those purchased from algae banks [4,11,16,17,48,74]. For instance, Zhou et al. [27] conducted a comprehensive analysis and comparison of the algae strains isolated from local areas with those purchased from algae banks when they were cultivated in swine manure wastewater and found that locally isolated algal strains tended to adapt to local environments better than the purchased strains. Likewise, Pérez et al. [74] and Zhou et al. [16] observed increase in growth rate and nutrient removal efficiency when microalgae were cultivated on wastewaters where they were isolated, probably due to progressive acclimation of these selected indigenous microalgae strains [11,48,74]. Another approach is to select microalgae consortium (a mixed culture of different wild algae species) because it was found that the microalgae consortium performed better than monoculture in terms of nutrient removal and biomass productivity [25,59].

Resistant strains can be obtained through genetic engineering and/or breeding manipulation in order to obtain extra resistance to environment stress and/or improve oil synthesis [98,99]. For example, Malik [75] reconstructed microalgae strains by genetic manipulation to improve cell acclimation to progressively higher pollutant concentration. Another researcher genetically engineered the microalgal strain *Cyclotella cryptic* for enhanced production of biodiesel fuel [76]. Genetic and metabolic engineering may have the greatest impacts on improving the economics of production of microalgal biodiesel in the near future [12,77–79].

Finally, from engineering aspect point of view, the selected promising microalgal strains must satisfy the demand of ease of scaling-up in industrial cultivation system and ease of harvesting through natural aggregation/bioflocculation, etc., which will be discussed in detail in the next following sections.

In summary, the ideal candidate wastewater-grown microalgae should have following characteristics: (1) fast growth; (2) high oil content; (3) high resistance to contamination for different type of wastewater; (4) high tolerance to variation of local climate as well as operating conditions; Finally, screening robust wild type strains from local environment and constructing engineered microalgae with desired characteristics may be particularly important and deserve further investigation for advanced wastewater-based algae cultivation system.

4.2. Cultivation systems

Cultivation systems reported in the literature include raceway ponds with paddle-wheel agitation, multi-layer open pond-like bioreactors and different types of closed bioreactors such as tubular bioreactor, flat panel bioreactor, coil bioreactor, bag bioreactor, floating bioreactor, Fermenters, and solid media surface cultivation bioreactor [12,16,29,30,37,43]. Fig. 2 shows images of the most common bioreactor configurations. Wastewater treatment plants may employ high stabilization pond, lagoon, and aerated ponds, where algae may also be cultivated. Table 2 makes a comparison between the open and closed bioreactors concerning the production of wastewater-grown microalgae. In order to treat large amounts of wastewater generated from sewage, industry and agriculture, bioreactors should be easily scaled up, and operated for efficient nutrient removal. From this point of view, multi-layer bioreactor and raceway pond with paddle-wheel were considered as most feasible and cost-effective culture systems for treatment of various types of wastewaters (Table 2) [30,37,43].

Most of published data using wastewaters in the literature were obtained from experiments conducted in small lab scale with optimized cultivation conditions such as optimized light intensity and temperature. Only a few studies demonstrated the feasibility of large-scale wastewater-based microalgal production in outdoor environment. For instance, large scale production of algal biofuels using wastewater in high rate shallow, open raceway algal ponds, was used for treatment of municipal wastewaters [30,33,80]. A 2000 L and 40,000 L pilot-scale multi-layer bioreactors were successfully developed [37,39] and were used to cultivate microalgae on centrate and animal manure wastewaters for effective algal biomass production and efficient nutrient removal. This multi-layered structure renders the system a very small footprint, and therefore it is feasible to co-locate such small footprint system with local municipal wastewater treatment plants or animal farms where spare land is limited (Figs. 2E and 3). The open shallow

Table 2
Comparison of properties of different bioreactor systems.

Bioreactor type	Scaling-up feasibility	Cost	Land requirement	Growth rate	Light efficiency	Contamination issue
High-rate pond	Easy	Low	High	Low	Low	High
lagoon	Easy	Low	High	Low	Low	High
Multi-layer bioreactor	Easy	Middle	Low	High	Middle	Middle
Coil-bioreactor	Difficult	High	Low	High	High	Low
Air-lift bioreactor	Difficult	High	Low	High	High	Low
Bubble column reactor	Difficult	High	Low	High	High	Low
Tubular reactor	Difficult	High	Low	High	High	Low
Bag reactor	Middle	Low	Low	High	High	Low
Floating reactor	Easy	Low	Low	High	High	Low

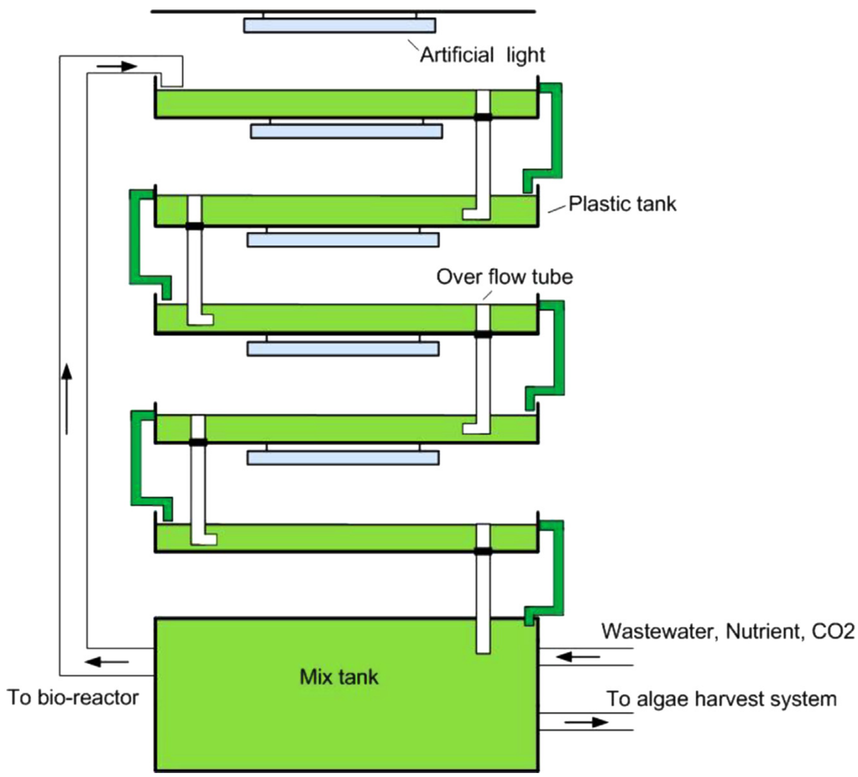

Fig. 3. Design scheme of the newly developed multi-layer bioreactor system (20,000 L pilot scale multi-layer bioreactor located in the Rosemount Research and Outreach Center (Rosemount, MN, USA). August, 2012).

trays significantly reduce the impact of wall fouling on light transmission, and the system maintenance (cleaning) is minimal [37,39,43]. Recently, Wiley et al. [81] reported a novel microalgae cultivation system, named offshore membrane enclosures for growing algae (OMEGA) system (Fig. S2), in which microalgae were cultivated in floating photobioreactors deployed in protected bay marine environment (located in protected bays near coastal cities) with municipal wastewater outfalls and sources of CO_2-rich flue gas on shore. The advantages of the OMEGA system included uniform temperature maintenance due to floating on the ocean, low cost fertilizer input due to nutrient-rich wastewater and CO_2-rich flue gas addition and low cost energy input for mixing due to the agitation provided by ocean waves, etc. [81]. However, fouling on both sides of the membrane will likely occur. If this issue is not addressed, further development of the technology will be hindered.

In summary, Wastewater-based algal production systems need to be further improved in order to become more competitive and more economically feasible.

4.3. Environmental factors

Although the advantages for wastewater-based algae cultivation system are significant as discussed previously, its operation is not trivial and requires close attention. In this section, we will review and discuss how algae growth is affected by various environmental factors.

The key environmental parameters includes light, temperature, pH, predation by zooplankton, pathogens (including bacteria, fungi and viruses) and invading species competition. The climate conditions include daily variation of solar radiation and temperature. In tropical and sub-tropical region with relatively high solar radiation and temperature, algae may grow to high density [11,82,83]. The influence of light-saturation on algae growth in wastewater-based algae cultivation system is minimal due to the high turbidity of wastewater [4,5,26,27]. Different strains respond to light intensity differently. When cultivated on concentrated wastewaters, *C. protothecoide* was able to grow fast at light intensity up to 200 μmol $m^{-2}\,s^{-1}$ while the growth of *C. kessleri* was inhibited when light intensity exceeded 120 μmol $m^{-2}\,s^{-1}$ [36]. pH of the culture broth affects the CO_2 availability and nutrient ions concentration, algal respiration, the alkalinity and ionic composition [4,15,84,85]. As described previously, pH of the media increases during algae cultivation due to depletion of CO_2 and HCO_3^- by photosynthesis. In addition, previous researches [15–17,86,87] reported that an increase in pH of culture broth may result in algae coagulation and adsorption of inorganic phosphates [15,16,31]. Heterotrophic oxidation of organic compounds by bacteria may also affect CO_2 availability [80,84,88]. However, domestic sewage typically contains insufficient carbon to sustain algae growth [59,89]. CO_2 addition has been shown to enhance algal productivity in wastewater-based algae cultivation system [17,37,59,61] and CO_2-rich flue gas from power plants could be another option for extraneous CO_2 supplement.

Wastewater-based algae cultivation is susceptible to other factors such as grazing by herbivorous protozoa and zooplankton (e.g., rotifers and cladocerans) which can reduce algal concentration and even cause culture crash in 2–3 days [90,91]. For example, rotifers and cladocerans at high densities ($>10^5$/L) were shown to reduce algal concentrations by 90% due to Daphnia grazing over several days. Fungal parasitism and viral infection can also significantly reduce the algal population in a pond within a few days and trigger changes in algal cell structure, diversity and succession [30,92]. Liu et al. [93] found out that the concentration of virus particles from activated sludge ranged from $0.28\times10^9\,ml^{-1}$ to $27.04\times10^9\,ml^{-1}$ and indigenous viruses are abundant and dynamic in the municipal treatment system and may play an important role in functioning of the system. One option to tackle these issues is to increase the initial inoculums of microalgae [35]. By this way, algae could compete with these predators

and pathogens as well as invasive algal species and thus inhibit their growth. And high initial concentration of algal cells could help shorten the retention time of wastewater-based algae cultivation system [15,35,36]. Another alternative is to keep 10 ppm chlorine in culture every 3 days to prevent culture crashes (Private discussion with lab members of Prof. Qiang Hu at Arizona State University). Overall, influence of the microbial community in different types of wastewaters is complex and deserves further investigation.

4.4. Operational parameters

Agitation, exogenous carbon supplementation, and harvest frequency or hydraulic retention time (HRT) are key operational parameters which affect algae growth, biomass productivity, and nutrient removal significantly. The traditional agitation methods for algae culture include bubbling, rotation, pumping and paddle-wheel based mixing, depending on bioreactor type. For tubular bioreactor, the pump is commonly used for mixing [15,16,71]. For vertical flat bioreactor, bubbling is an efficient mixing way for algae cultivation [91]. Paddle-wheel based mixing system is adopted for outdoor large-scale cultivation such as typical raceway open pond [30,33,94].

Exogenous carbons may be added to the wastewater if the original carbon source has been mostly consumed while other nutrients (e.g., N and P) are still sufficient, or a second growth mode is desirable. For example, the two-stage cultivation mode developed by Zhou et al. [17] includes a first heterotrophic dominated cultivation in which algae growth relies mainly on organic carbons present in municipal wastewater (centrate), followed by a second autotrophic dominated cultivation in which CO_2 is supplemented to allow continue assimilation of N and P by algae. The maximal biomass concentration and lipid content at the first and second stages reached 1.12 g/L and 28.90%, and 1.16 g/L and 33.22%, respectively, and the nutrient removal efficiencies for TN, TP, NH^4-N and COD at the end of the two-stage cultivation were 90.60, 98.48,100 and 79.10%, respectively. Co-locating an algae based treatment system with an existing wastewater treatment facility where flue gas is available from sludge combustion is particularly beneficial (Fig. S1). The heat and electricity generated from the sludge combustion process can be used for the conventional wastewater operations as well as algae cultivation, harvest, and processing [4,17,95–97].

HRT is commonly referred to the average period that a given quantity of input biomass remains in the constructed bioreactor. HRT had a large impact on total biomass and lipid productivity and nutrient removal efficiency in wastewater-based algae cultures [15,16,30,33,37]. For nutrient-rich wastewaters such as centrate described above, the 1/3 HRT was considered optimal for maximal algae growth and nutrient removal in 25 L Coil bioreactor (Fig. 3D) [15,16,37].

4.5. Harvest

Lack of an efficient and cost-effective algal biomass harvesting technology is another key limiting factor impeding the commercial algal biofuel industry and microalgae-based wastewater treatment. In general, harvesting of algae from culture broth accounts for at least 20–30% of the total costs of algal biomass production due to their tiny cell size (< 70 μm) and strong negative charge on the cell surface. A variety of harvesting and dewatering technologies have been extensively studied including centrifugation, flotation, flocculation, filtration, sedimentation, or combination of above methods (Table S1) [98]. Among these processes, centrifugation is considered to be the most efficient method [99]. However, high capital cost, energy input and operational cost impede its large scale application and currently is only used to harvest microalgal cells containing high-value bio-products such as PUFA, and cosmetics and other commodity materials in small-scale. The main disadvantage of flotation is its environmental and economic viability (Table S1). Flocculation is conducted by addition chemical/synthetic polymers to the broth before harvesting. The economic viability and potential environmental safety issues caused by these polymers limit the value of this technology [98].

Table 3
Lipid content and lipid productivity of robust microalgae species grown in different wastewater resources.

Wastewater type	Microalgae species	Biomass productivity ($mg\ L^{-1}\ d^{-1}$)	Lipid content (% DW)	Lipid productivity ($mg\ L^{-1}\ d^{-1}$)	Reference
Carpet mill	*Chlorella asccharophila*	23	18.10	4.2	[25]
Carpet mill	*Scenedesmus sp*	126.54	12.80	16.2	[25]
Dairy wastewater,25X	*mix-culture of chlorella sp., Micractinium sp., Actinastrum sp*	NA	29.00	17	[59]
Primary clarifier effuent	*Mix-culture of chlorella sp., Micractinium sp., Actinastrum sp*	NA	9.00	24.4	[59]
Second effluent	*Scenedesmus sp.* LX1	9.2	31–33	8	[48]
Activated sludge extract	*Chlorella pyrenoidosa*	11.55	NA	NA	[131]
Digested sludge extract	*Chlorella pyrenoidosa*	51.82	NA	NA	[131]
Settled sewage extract	*Chlorella pyrenoidosa*	275	NA	NA	[31,32]
Activated sewage wastewater	*Chlorella pyrenoidosa and scenedesmus sp*	92.31	NA	NA	[31,32]
Secondarily treated sewage	*Botryococcus braunii*	35.00	NA	NA	[70]
Artificial wastewater	*Scenedesmus sp*	126.54	12.80	16.2	[132]
Centrate	*Auxenochlorella protothecoides*	268.8	28.9	77.7	[16]
Centrate	*Chlorella sp*	120.8–241.7	17.41–26.99	21.0–94.8	[4]
Centrate	*Chlamydomonas reinhardtii*	2000	25.25	505	[71]
Centrate	*Heynigia sp*	210.4	24.16	50.8	[4]
Centrate	*Micratinium sp*	231.4	18.41	42.6	[4]
Centrate	*Hindakia sp*	275.0	28.30	369	[4]
Centrate	*Scenedesmus sp*	193.8–247.5	25.70–30.09	49.8–74.5	[4]

Filtration method is only used for harvesting microalgae with long length or formation of large-colony (e.g., *Spirulina sp.* and *Micractinium sp.*) [99]. Recently, Zhou et al. [17] reported a natural metal ion mediated self-sedimentation/flocculation method by growing algae on metal ion containing wastewaters, which could be another option for cost-effective harvesting algal biomass for biofuel purpose.

Another alternative is to immobilize or entrap microalgae cells in suspended media. The general techniques for immobilization of microorganism (e.g., bacteria, microalgae, yeast, and fungi) are to mix these microorganisms with synthetic (e.g., acrylamide, polyurethane, polyvinyl, and resins) and/or natural polymers (e.g., alginate, carraggenan, agar, and agarose) [100]. However, these polymers are too costly and hence limit their applications in large-scale [101]. Instead of using synthetic polymers, Zhou et al. [5,102] co-cultured pellet-forming filamentous fungi with microalgae, resulting in large size (typically 2–4 mm in diameter) of microalgae and fungi pellets, which can be easily removed from the culture broth through simple filtration (Fig. S3). Alternatively, the pellets, where microalgae are immobilized and stabilized, may remain in the culture broth to perform stable functions if so desired, i.e., wastewater treatment and high-value products production [5].

4.6. Utilization of wastewater grown algae

The algal biomass produced and harvested from wastewater treatment process promise a wide range of the renewable fuels and value-added products through various pathways. In this section, the potential applications of wastewater grown microalgae for various biofuels and value-added products will be reviewed and discussed.

4.6.1. Biofuel applications

Oleaginous microalgae-based biodiesel, as biodegradable and clean-burning renewable energy source, has attracted widespread attention. However, lack of stable low cost feedstock supply is the main bottleneck hindering the development of biodiesel production worldwide [103]. Numerous researches demonstrated that microalgae have definite advantages over conventional oil-crops based biofuel sources. However, the economic viability remains the key obstacle to the commercialization of algae-based bio-energy production [4,16,102]. Wastewater-grown microalgae are considered as potential feedstock for biodiesel production [11,15,16,17]. For example, a study conducted by Chinnasamy et al. [25] using mixed wastewaters containing carpet industry effluents (85–90%) and municipal sewage (10–15%) demonstrated that approximately 0.40–0.78 t ha^{-1} $year^{-1}$ of algal biodiesel could be produced. However, the lipid content of the wastewater-grown algae was only 6.82%, much lower than the average algae content of 17–25% probably due to the intrinsic characteristics of wastewaters (rich in N and P, which are not suitable for high lipid accumulation) (Table S2 and Table 3). Alternative, growing microalgae on organic carbon-rich wastewaters such as centrate and waste molasses might be a good solution since maximal lipid contents of algal biomass could be as high as 33.53% and 57.6%, respectively [4,47], although issue of availability of this type of wastewater should be addressed before wide use is anticipated. The lipids derived from microalgae grown on organic-rich wastewater can be used to produce other renewable or green diesel products such as Bio-gasoline, Bio-jet fuel, and green diesel by a process known as catalytic hydro-processing [2]. The main disadvantage of above mentioned biofuels is the relatively high oxygen content and nitrogen content compared to fossil oil-based fuels. Therefore, further investigation to reduce the oxygen and nitrogen in the final biofuel while maximizing the final energy content is greatly needed.

Another alternative way to fully utilize wastewater-grown microalgae biomass is to produce biogas or biohydrogen through anaerobic digestion of whole algal biomass especially for those having low lipid contents directly in a sealed anaerobic bioreactor, which could be one of the practical strategies from the energy recovery efficiency point of view compared with other conversion technologies [104]. Other alternative and simple ways to utilize wastewater-grown microalgae biomass is to produce bio-crude oil via direct thermochemical conversion, such as pyrolysis,

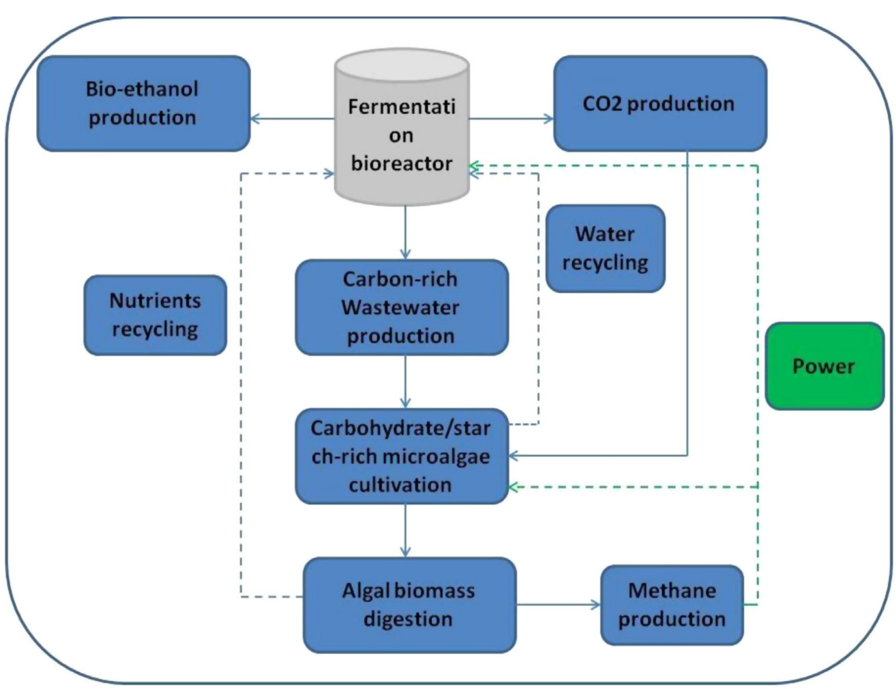

Fig. 4. Integrated wastewater algae based bioethanol production process diagram.

gasification and liquefaction [95–97,104–107]. By these conversion processes, wet algal biomass after harvested can be directly gasified or hydrothermally liquefied, resulting in significant energy saving.

Carbohydrate/starch-rich microalgal biomass also can be used as feedstock for bio-ethanol production through fermentation processes [108–110]. For instance, Valderrama et al. [111] reported the feasibility of accumulation of carbohydrate/starch for microalgae grown on organic rich industrial effluent, especially for ethanol and citric acid production. And Selecting starch-rich microalgae species for this type of wastewater treatment have dual purpose of wastewater treatment and bio-ethanol feedstock production. An integrated process to combine the benefits of each process should be developed and optimized in the near future (Fig. 4). Moreover, Ellis et al. [20] demonstrated an integrated fermentation production strategy to produce acetone, butanol, and ethanol, simultaneously, by *Clostridium spp.* using wastewater-grown algal biomass as sole carbon source. Their research results showed that 9.74 g/L of total ABE was produced under optimized conditions, which suggested an alternative high energy fuels and chemical production using wastewater-grown microalgae. Besides traditional fermentation, metabolic engineering work to introduce a new ethanol fermentation pathway for microalgae in order to photoautotrophically convert CO_2 to bioethanol was recently reported [76,112]. This opens up a new vista for sustainable production of a variety of biofuels via photosynthetic microalgae.

4.6.2. Non-fuel applications

Wastewater grown microalgae could also be used for non-fuel applications, such as chemicals, fertilizer, biopolymers, bioplastics, paints, dyes, colorants, lubricants, cosmetic, pharmaceutics, nutritious food and animal feed, pollution control (CO_2 sequestration, uranium/plutonium sequestration, fertilizer runoff reclamation, sewage & wastewater treatment, etc.). For example, Mulbry et al. [113] reported an alternative to traditional land spending of animal manure by recycling manure nutrient such as N and P through converting the N and P into algal biomass as a slow release fertilizer (replacing commercial fertilizers for crops).

It is worth noting that animal wastewaters such as swine and dairy manure contains much lower metals than industrial and municipal wastewaters, and anaerobic digestion will further precipitate metals and kill potential pathogens. Thus the potential of toxicity for animal and fish as well as human consumption will be minimal if not entirely eliminated compared with industrial and municipal wastewaters [4,5,26,27]. It is therefore feasible to grow microalgae in animal wastewaters for the purpose of producing safe animal and fish feed ingredients. For example, in aquaculture, traditionally, it has heavily depended on fish meal to meet their critical protein requirements and fish oil for omega-3 fatty acid requirements. Some microalgae species contain high-grade protein source when grown on nitrogen-rich animal wastewaters, with almost all species containing similar amino acids composition and rich in the essential amino acids [50] and low in ash content [5,26,27,114]. Some microalgae may be excellent source of polyunsaturated fatty acids (PUFAs) such as EPA (C20:5n-3) and DHA (C22:6n-3) (Table S3) [4,27,114]. These unique nutritional profiles for wastewater grown microalgae make them ideally fit for aquafeed and meet the need of growing aquaculture [115].

4.6.3. System integration for algae based bio-refinery

Although promising, growing algae on wastewater for biofuel purpose still faces many challenges before the industry becomes technologically and economically viable in the near future.

One solution to these challenges is to develop processes to significantly improve the nutrient assimilation efficiency and enable maximum recycling/reuse of water to minimize or entirely eliminate external inputs of fertilizers and fresh water. Additionally, a biorefinery concept, which has been defined as “the sustainable processing of biomass into a spectrum of marketable products and energy” [116,117], should also be considered and incorporated into wastewater-based algal biofuel production systems. In this context, the biorefinery strategy was defined as a

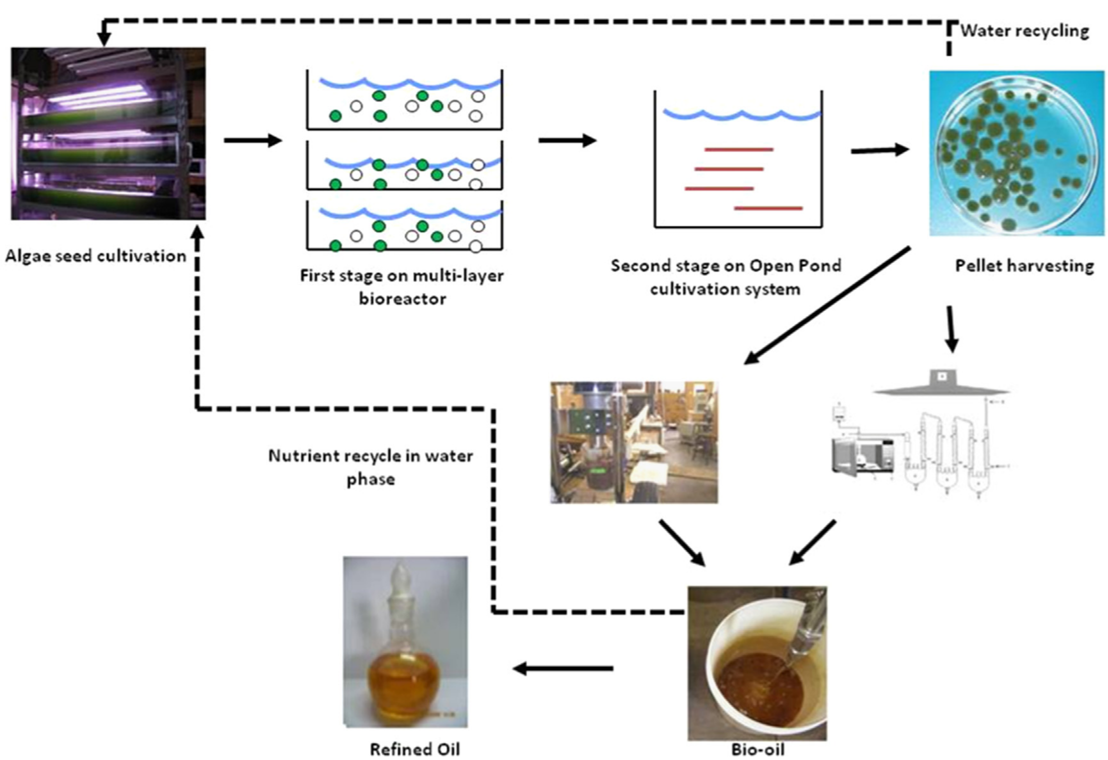

Fig. 5. Integration system of wastewater-based algae platform for biofuel and biobased products.

platform that integrated wastewater-based algal biomass conversion processes and facilities to produce fuels, power, value added byproducts from microalgal biomass and at same time for wastewater treatment [17,118,119]. Taking into consideration all the innovative technologies and methods mentioned above, the flow chart for an integrated scheme to reuse the carbons and other nutrients in the water and solid phases of hydrothermal liquefaction and pyrolysis processes of algal biomass is described and shown in Fig. 5. Multi-layer pond-like bioreactor was used to cultivate selected robust microalgal strains on different types of wastewaters for low cost algal biofuel feedstock production and simultaneous effective wastewater treatment. In harvesting stage, newly developed filamentous fungi-based harvesting method is incorporated and eventually algal pellets are obtained, which can be easily removed using simple filtration process, during which water is clarified and recycled back to the cultivation facility. The hydrothermal liquefaction and pyrolysis processes are used to convert harvested algal biomass to liquid fuels with some byproducts containing carbons and other nutrients which can be recycled for algae production. Previous studies showed that the aqueous phase and solid chars after pretreatment of algae whole cell biomass through hydrothermal liquefaction and microwave-assisted pyrolysis contain large amount of nitrogen, phosphorus, and carbons, which could be reused by algae (Table S4). It is expected that about 45% of C and 70% of N can be recycled for algal cultivation [119]. High concentration of phenols and Ni may be toxic to algae; however, when they are added to the large volume culture broth, they will be diluted and hence may not pose serious threat to algal cells [119].

5. Economics and life cycle analysis

5.1. Cost analysis

For bioenergy production from algal biomass, the main obstacle for commercialization is its high capital and operating costs. Several research groups evaluated the economics of both open pond and photobioreactor (PBR) systems in order to better understand the current state of algal biofuel technology as it stands today, and to identify the most significant opportunities for cost reduction in the near future. For example, a comprehensive techno-economic analysis conducted by Davis et al. [120] showed that the cost of lipid production of \$8.52/gal and \$18.10/gal were achieved for open pond and PBR systems, respectively, by using 25 g/m^2/day (open pond) and 1.25 kg/m^3/day (PBR) algal biomass productivity and 25% of lipid content as a baseline, bringing the final cost of diesel determined to be \$9.84/gal for open pond and \$20.53/gal for PBR, respectively. Similar results were also reported by Abayomi et al. [121] using different scenarios for algal biomass production in British Columbia. Given the current fossil-based diesel production cost of \$2.60/gal [120], above mentioned results further demonstrated that the price of current microalgae-based biofuels could not be competitive with traditional fossil fuels if algae-to-biofuels facilities were to be constructed in large-scale at present. The reason for current high cost of algal biofuels production was partially due to the cost of nutrients such as fertilizer (N, P and other trace elements) and freshwater for microalgae cultivation, which account for 20–30% of the total cost of whole algal biodiesel processes [23,91,122]. Recent studies also suggested that integrating algae biomass production with wastewater treatment is the only viable solution to reduce economic burden and increase sustainability of algal biofuels at commercial scale [4,11,16,23]. For example, the required nutrient and freshwater can be supplied by using wastewater, the cost in cultivation part could not only be eliminated, but the wastewater treatment credit can be redeemed also. In addition, much higher biomass and lipid productivity achievable in organic-rich wastewater-based algae cultivation system through mixotrophic cultivation could further increase the economics of such algae production system [4,47]. Therefore, the cost could be further reduced up to 50%, which makes the wastewater-based biofuel more comparable to petroleum-based diesel fuel. There is still more room for substantial improvement potential in wastewater-based algal biofuels economics through fundamental biological breakthroughs and engineering innovation for both open pond and PBR cases.

5.2. Environmental cycle analysis

Besides the economic benefit, another advantage of wastewater-based algae cultivation is the significant reduction of ecological footprint. Some recently published life cycle analysis (LCA) researches have also confirmed the environmental sustainability of the wastewater-based algal biofuel production processes. For example, Clarens et al. [23] conducted a LCA study of microalgae, switchgrass, corn and canola and suggested that utilization of nutrient-rich wastewater instead of freshwater and fertilizers could offset most of the environmental burdens associated with microalgae production. Another LCA study by Yang et al. [123] demonstrated that approximately 90% of freshwater could be saved when wastewaters were used to cultivate microalgae while the required N was reduced by 94%, and the need for added potassium (K), magnesium (Mg), and sulfur (S) from fertilizer was reduced 100% by replacing freshwater with wastewater. Clarens et al. [23] further modeled wastewater-based algal biofuel production system using three types of waste streams and compared to those in a freshwater-based open pond system. The results showed that all three models significantly improved the life cycle burden on the algal biofuel production and when urine was modeled as the wastewater resource, the algal-based process was shown to be more environmentally beneficial than terrestrial plant biofuel crops.

However, there are many variations and uncertainties faced by wastewater-based algal biofuels [124,125]. The wastewater sources, nutrient profiles, infrastructure and locations, and pretreatment methods, could potentially increase the difficulty and uncertainty in algal biomass production [124]. For example, the nutrient profile of wastewater from some sources may be unsuitable for algae cultivation due to low nutrient levels, mismatched carbon to nitrogen ratio, or the presence of inhibitors, which could result in poor algal lipid productivity and significantly reduce nutrient assimilation efficiency [126]. Therefore, costs related to downstream process might be increased accordingly.

In a recent LCA study on environmental impacts of wastewater-based algal biofuels, Mu et al. [127] compared different pathways include: (1) different nutrient sources, such as centrate, swine manure, and freshwater with synthetic fertilizers; (2) algae cultivation methods, photobioreactor and open pond, and (3) biomass conversion technologies, e.g., hydrothermal treatment, microwave pyrolysis with hydro-upgrading, combustion, and lipid extraction with transesterification. The results confirmed that the environmental performance of wastewater-based algal biofuels is generally better than freshwater-based algal biofuels. However, the performance is largely dependent on the nutrient profile of the wastewater and subsequent downstream process. Since the centrate contains optimal nutrient level for algal growth, the centrate based PBR system out performed swine manure based system. For oil extraction and conversion process, wet lipid extraction is more suitable for high lipid content algal biomass while pyrolysis process is more favorable to lower lipid content algal feed. However, the availability of a suitable wastewater sources limits the potential for large-scale implementation of the system. Thus, it

is still difficult for algal biofuels to provide a large-scale and environmentally viable alternative to petroleum transportation fuels without considerable improvement in current production technologies.

6. Concluding remarks

The technical feasibility of many algae production technologies has been extensively investigated and demonstrated. However, the economic viability and environmental sustainability remain the key obstacles to the commercialization of these technologies. Many of these challenges are cost-associated, and cannot be overcome without technical breakthroughs and innovative system integration. Coupling algal biofuel production with wastewater treatment is considered as the most viable solution to this issue due to its advantages over traditional algae farms, including: (1) cost effectiveness: the traditional commercial algal production requires a large amount of freshwater, fertilizer and carbon dioxide which accounts for approximate 30% of total production costs, while most of these costs could be saved using waste streams from municipal, industrial and agricultural wastewaters, which could provide the needed water and nutrients for algae growth. Moreover, CO_2 could be provided partially by bacterial oxidation of wastewater organics and onsite exhaust gas; (2) easy harvesting: many of the algal strains in wastewater treatment processes form large colonies (50–200 μm), and cell aggregation may be achieved through nutrient limitation or CO_2 addition or natural present metal ions-based self-sedimentation, which further lower the cost of algae harvesting; (3) GHG emission reduction: the US Environmental Protection Agency (EPA) has specifically identified conventional wastewater treatment plants as major contributors to greenhouse gases. In algae based wastewater treatment, algae consume more CO_2 than the treatment process releases, making the whole process carbon negative. In addition, carbons in the wastewaters are sequestered, which brings additional environmental benefits and potential carbon credits; (4) Credits for wastewater treatment: algae-based wastewater treatment is considered to be more cost-effective than traditional activated sludge process and other secondary treatment processes in removing BOD, pathogens, P and N. In addition, heterotrophic bacteria in the wastewater streams help remove high concentration of oxygen produced during algae photosynthesis, which is one of the key factors inhibiting algae growth in closed bioreactor systems. Moreover, microalgae-based wastewater treatment has other advantages such as reduced sludge formation compared with traditional wastewater treatment process. Also, the resulting sludge with algal biomass is energy rich and can be further processed to produce biofuel or other valuable products such as fertilizers. Finally, the high cost of algae-biofuel arises from intensive energy for algae harvesting and downstream processing for traditional algal farm could also be reduced significantly by using advanced wastewater-based cultivation system and process integration. The integrated approach described can improve wastewater nutrient removal efficiency, water and nutrient recycling, CO_2 fixation, and lipid yields, and lower algae harvesting and conversion cost. However, before the advanced wastewater-based algal biofuel production technologies could be widely implemented, more research is still needed to (i) better understand the mechanism of wastewater nutrients removal by microalgae; (ii) improve capabilities of microalgae to tolerate different types of wastewaters and environmental stresses through selecting locally isolated robust microalgae strains and/or genetic engineering; (iii) optimize the environmental parameters and combine heterotrophic and mixotrophic cultivation as a possible avenue of research for maximal algal biomass and lipid productivity and demonstrate at commercial scales; (iv) develop efficient and cost-effective algae harvesting and conversion technologies and integrated biorefinery processes for economical production of biofuel and chemical intermediates as well as nutraceutical and pharmaceutical products; (v) conduct techno-economic assessment to determine the economic viability of currently reviewed wastewater-based algae biofuel systems here and life cycle analysis to evaluate the water and carbon footprints and other environmental impacts on the system, and thus guide research on system integration and innovation in a more sustainable, economically viable and environmentally friendly way.

Conflict of interest disclosure

The authors declare no competing financial interest.

Acknowledgments

The study was partially supported by grants from the University of Minnesota Center for Biorefining, the Legislative-Citizen Commission on Minnesota Resources (LCCMR), and the China 863 Projects (2012AA021704), NSF of China (Grant 21177067), the Key Program of Natural Science of the Jiangsu Province of China (Grant BK2010034) and China International Cooperation Projects (2010DFB63750).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.rser.2014.04.073.

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